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THE INDUCTION OF MUTATION IN SYNCHRONOUSLY DIVIDING CULTURES OF SACCHAROMYCES CEREVISIAE BY ULTRAVIOLET IRRADIATION

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA

APRIL, 1966

ABSTRACT

Mitotic synchrony was induced by a starvation technique in a haploid adenine-requiring strain of Saccharomyces cerevisiae. Periodic samples taken from synchronous cultures were irradiated with a constant dose of ultra-violet light and plated to determine survival rates and rates of mutation affecting adenine biosynthesis. Microscopical observation of synchronized populations showed that the budding phase coincides with the period of peak sensitivity to induced mutation. Williamson and Scopes, using a similar system, have demonstrated that DNA synthesis occurs during the time of bud formation; therefore it is proposed that increased mutability is associated with the DNA replication process.

ACKNOWLEDGEMENTS

The assistance of Dr. Clayton Person, who supervised the course of this study, is gratefully acknowledged. I would like to thank Dr. J. Weijer and Mr. Philip Rhynas for their excellent advice on technical procedures. I would also like to thank Dr. G.W.R. Walker for his constructive criticism, and Miss Margaret Maclean who prepared the manuscript.

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INTRODUCTION

Demonstration of the effects of ultraviolet (UV) radiation on nucleic acid bases, synthetic polynucleotides and deoxyribose nucleic acid (DNA) *in vitro*, and the subsequent correlation of these effects with *in vivo* inactivation in bacterial and phage systems, has led to certain predictions about the mechanisms of action of UV on intact chromosomes (see Wacker, 1963, for review). These predictions suggest that genetic sensitivity should be related to volume and state of organization of DNA.

Saccharomyces cerevisiae is a yeast which has a replication and division cycle generally similar to that of the higher organisms (Williamson, 1964). It can be grown in cultures synchronized with respect to cell division and time of DNA replication (Williamson and Scopes, 1960). The use of a strain carrying an adenine-red genetic marker for the study of UV-induced mutation in synchronous cultures should permit an estimation of the amount of genetic change (mutation) induced by UV at the various stages of the cell cycle. Determinations of the lethal effects of UV for varying nutritional conditions and for different stages of the division cycle should further provide an indication of non-genetic, or general physiological factors which may act to modify genetic effects of UV.

REVIEW OF THE LITERATURE

I. Mechanisms of UV Radiation Effects

The chemical basis for biological UV inactivation has been subject to direct experimentation because of the specificity of action of UV radiation. In a recent review by Wacker (1963) it is proposed that thymine dimerization in DNA is the primary inactivation event induced by UV. He argues that intermolecular dimerization, a reaction which has a high probability of occurrence only during replication when polynucleotide strand separation occurs, may completely block cell division to produce a lethal effect. Inactivation in synchronous cultures of Enterococcus Stei has been positively correlated with thymine dimerization, and is maximal when DNA is in the single-stranded condition.

Mechanisms of UV mutagenesis, based on altered base-pairing properties of irradiated DNA, are also suggested by Wacker. Intramolecular thymine dimerization likely affects hydrogen-bonding capacity, and therefore "correct" pairing with adenine. Cytosine dimerization results in deamination to form a uracil-dimer photo-product with altered base-pairing properties.

Repair mechanisms have been demonstrated which remove UV-induced lesions from DNA. Monomerization of thymine dimers has been induced by an enzyme isolated from bakers' yeast (dark recovery) and by visible light (photoreactivation) (Patrick and Haynes, 1964; Rupert, 1961).

Doudney, in another review (1961), proposes an alternative mechanism for UV mutagenesis based on post-irradiation effects first observed by Witkin in E. coli. Witkin found that DNA synthesis with prior protein synthesis is required for the production of mutation, but the fixation of mutation is dependent only on RNA synthesis. Doudney therefore suggested that mutation is produced through a UV-modified RNA precursor which functions as an intermediate in an alternate form of DNA synthesis, or modifies DNA formed by a direct replication mechanism. Such a model would explain the phenomenon of delayed mutation and the production of sectored colonies mentioned by Witkin.

Recent work on the UV-induction of sectored colonies for adenine-red mutants in Saccharomyces cerevisiae has given evidence that the main targets are thymine-thymine sequences in the DNA of the gene, rather than nucleic acid precursors of RNA (Ito et al., 1964). The UV action spectrum observed for the sectoring effect was very similar to that of thymine. Photoreversion of the effect was induced to a maximum of 80%. This is interpreted as evidence against a delayed (precursor) effect, but is not directly contradictory to the mechanism proposed by Doudney. The same group, using X-ray mutagenesis in the same system, reported a multi-hit induction curve for whole-colony mutants, and a single-hit curve showing a saturation effect for fractional-colony mutants. It is suggested that both UV and X-ray produce whole- and fractional-colony mutants by a double- and single-strand change in DNA respectively (Yamasaki et al., 1964). More recent experiments with

E. coli provide further support for this model with the finding that UV-induced mutation and death are caused primarily by photo-reactivable damage of the same type, i.e. pyrimidine dimers, and are not due to repair mistakes since post-incubation with chloramphenicol has no effect (Kondo et al., 1965).

II. Relationship between Cell Stage and UV-Induced Lethality and Mutation

Elkind and Sutton (1959a) first demonstrated that haploid budding cells of Saccharomyces cerevisiae are relatively less susceptible to UV-induced lethality than are inter-divisional cells. The survival curve for non-dividing cells alone was found to correspond to the initial, sensitive portion of the survival curve for a mixed population. A resistant tail, correlated with the proportion of budded cells present, was characteristic of the mixed population survival curve. This kind of resistance appears to represent a general phenomenon. It is found after ionizing irradiation as well as UV irradiation, and exists in a wide variety of yeasts, and in E. coli as well (Beam, 1959; Swann, 1962).

Radioresistance of budding cells is unrelated to the process of bud formation as such, since it is found in haploid dividing cells of the fission yeast, Schizosaccharomyces pombe (Swann, 1962). Beam (1959) correlated the nuclear cytology with the radiation sensitivity of individual cells. He identified the stage between interphase and metaphase as being the most radio-resistant. Similarly, the earliest stage(s) of meiosis are associated with decreased radiosensitivity (Magni, 1959).

The cell-stage effect has been further associated with a number of genetic and cytoplasmic effects. Experiments with X-ray mutagenesis indicate that the induction of recessive lethals is very rare in budding cells (Beam, 1959), and very frequent in non-dividing cells (Magni, 1959). In direct contrast, budding cells are no more resistant to the induction of dominant lethals than are non-dividing cells. Dominant lethality may, in fact, account for all lethality in budding haploid cells (Mortimer, 1959). Budding cells also show an increased sensitivity to X-ray-induced prototrophy (Fritz-Niggli, 1962). A more rapid repair of nucleic acid damage in cells at this stage is demonstrated by an earlier and faster photoreactivation (Elkind and Sutton, 1959b).

In comparison with non-dividing cells, the resistance of budding cells is disproportionately reduced by pre-starvation, especially for required amino-nitrogen sources. This has led to the conclusion that protein synthesis is implicated. It has been suggested that the budding process involves the synthesis of a specific protein structure which stabilizes DNA against radiation damage by reducing energy transfer, or by favoring repair processes (Moustacchi, 1963). In an excellent review on the radiobiology of yeast, James and Werner (1965) state that, "Investigations so far have failed to provide a generally accepted explanation of the cell stage effect."

III. Correlation of the Time of DNA Synthesis with Budding in Saccharomyces cerevisiae

The hypothesis underlying the present study is that the budding phase of S. cerevisiae is equivalent to the period of DNA synthesis in a synchronous system. It is therefore essential to establish the evidence for this hypothesis. Two basically different types of experiment, both done with S. cerevisiae, give mutual support to the presumed equivalence of the budding and DNA synthetic periods:

1. DNA extractions from synchronized cultures: both "natural" synchrony (stationary phase culture) (Ogur et al., 1953) and starvation-induced synchrony (Williamson and Scopes, 1960) have been studied. In both systems, DNA duplication (determined from warm perchloric acid extractions) was coincident with the emergence of buds.

2. Measurement of uptake of radioactive adenine in randomly dividing cultures by autoradiography (Williamson, 1965): after brief exposure to ^{3}H-adenine and extraction of non-DNA label, only cells synthesizing DNA during exposure were labelled. Position in the cell cycle was estimated by measurement of cell sizes. Results showed that adenine uptake and incorporation occurs during the first 27% of the cell cycle, which is occupied by bud formation. This is consistent with results from synchronous cultures.

MATERIALS AND METHODS

I. Organism

The haploid strain JA 29 of Saccharomyces cerevisiae, which was kindly supplied by Dr. R. K. Mortimer, was used throughout. This strain carries the genetic markers is_1 and ad_2, indicating requirements for isoleucine and adenine respectively. The ad_2 mutation is responsible for the production of a red pigment on appropriate solid medium.

II. Media

(a) Complete medium (YEP) (Roman, 1956)

1% yeast extract (Difco)
2% bactopeptone
2% dextrose, solidified with 2% agar where required

(b) Pre-starvation medium (Sylvén and Thorell, 1964)

0.05% yeast extract (Difco)
1% dextrose
phosphate buffer pH 6.5, minerals
supplemented with 20 mg/l adenine and 60 mg/l isoleucine

(c) Starvation medium (Sylvén and Thorell, 1964)

7.84 gm/l succinic acid
5.06 gm/l potassium hydroxide

mineral solution ($CaCl_2.6H_2O$ 5.0 g, $MgSO_4 . 7 H_2O$ 25.0 g, NaCl 5.0 g, H_3BO_3 0.025 g, $CuSO_4 . 5 H_2O$ 0.002 g, Kl 0.010 g, $FeCl_3 . 6 H_2O$ 0.010 g, $Na_2MoO_4 . 2 H_2O$ 0.010 g, $ZnSO_4 . 7 H_2O$ 0.020 g, $MnSO_4 . 1 H_2O$ 0.020 g, water 500 ml. Use 1 ml/100 ml medium.)

(d) Selective YEP medium

This medium, containing 1% yeast extract (Difco), 2% bactopeptone, and 10% dextrose, was found to induce intense pigment production, and was developed to facilitate the identification of adenine mutants.

III. Maintenance of Stock Cultures

Stock cultures were maintained on YEP agar at 4° C. Transfers were madeevery four to six weeks.

IV. Synchronization Procedure

The technique used to induce mitotic synchrony was that developed by Sylvén and Thorell. It utilizes a starvation pre-treatment to bring the cells to a uniform pre-division state. The method was standardized to the following schedule:

(a) A small loopful of cells from a single colony isolate was inoculated to 1 ml of pre-starvation medium in a dilution tube. This culture was incubated 24 hours to provide a good growth of cells. All incubations were carried out at 30° C.

(b) 0.1 ml of this culture (about 10^6 cells) was inoculated to 10 ml pre-starvation medium in a 250 ml flask and incubated 24 hours in a New Brunswick Model R27 shaker. Final cell concentration was about 3×10^7 cells/ml.

(c) The cells were centrifuged from the medium and washed twice in phosphate buffer. All centrifugations were done at 1,000 rpm for 5 minutes in a clinical centrifuge. This procedure selectively removed small cells from the population. Small cells have been shown to have a characteristic and different growth curve (Williamson and Scopes, 1960).

(d) The washed cells were resuspended in 10 ml starvation medium and incubated in shake culture for 5 hours. No budding occurred during this period; paired cells tended to separate. At the end of the starvation phase, most cells were uniform in size and round in outline.

(e) Centrifugation from medium and washing was repeated as in (c) above. The culture was resuspended in 10 ml pre-starvation medium and returned to the shaker. This point was taken as zero time in the synchronous phase, which was followed for three hours. Usually two or three 10 ml cultures were pooled at this time to provide an adequate volume for sampling. Cell concentration during this phase was 1-3 x 10^7/ml.

V. Cell Counts

Aliquots of 1.0 ml were removed at 15 minute intervals throughout most of the synchronous phase and fixed by quick freezing. This preserved the cells in their original state without any observable change in microscopic appearance. Cell counts were made within 24 hours by thawing these samples and immediately counting with a Spencer Bright-Line hemocytometer. About 1,000 cells in 320 small squares were counted for each determination, using a Leitz Ortholux microscope fitted with a phase contrast X40 objective and X10 periplan eyepieces. An arbitrary criterion was established for distinguishing budded from paired cells. This convention was based on the absence (in a budded cell) or presence (in a paired cell) of a cell wall between the adjoining cells.

VI. Irradiation

The ultraviolet light source was a G.E. 15 watt germicidal lamp emitting 80% of its ultraviolet light at 2537 Å. Samples taken from the synchronously dividing culture were diluted to about 10^6 cells/ml. A 10 ml aliquot of each sample was placed in a glass petri dish. This was agitated to maintain suspension during a 60 second exposure at a distance of 25 cm from the ultraviolet light source. The UV dose rate was estimated to be 400 - 450 ergs/cm^2/sec. This was measured by a G.E. UV meter, Model SM-200 with a Model WL-773 phototube (sensitivity range, 2000 - 3675 Å; maximum sensitivity at 2550 Å).

VII. Plating Experiments

(a) Control samples, taken at 15 minute intervals, were diluted serially to a final factor of 10^{-5}. Aliquots of 0.1 ml were spread on a series of three YEP agar plates for each sample, giving a count of 200 to 600 colonies per plate. All colony counts were done with a New Brunswick automatic counter.

(b) Samples for irradiation were taken on the same schedule as control samples. Following irradiation, these samples were immediately diluted and plated in triplicate series on YEP agar to yield 100 to 600 colonies per plate for irradiation-survival determinations.

(c) To obtain counts of radiation-induced mutation, 50 to 250 viable cells per plate were spread on selective YEP medium. Mutants appeared as red-pink and red-white sectored colonies as well as wholly pink and wholly white colonies among a background of red colonies.

EXPERIMENTAL RESULTS AND DISCUSSION

I. The Synchronous System

After five hours of starvation treatment, the number of paired cells was reduced from about 35% to 11 or 12%; budded cells were reduced to less than 1% of total cells. The cells were of a uniform size, round, and vacuolate. Viability, calculated by comparing hemocytometer counts with colony counts, was about 85% (see Appendix 1).

When the synchronized cells were suspended in growth medium they entered a lag phase lasting about 90 minutes (see Table 1 and Fig. 1). Plating efficiency rose from 85% to 95% during this period (see Appendix 1). Budding was nearly exclusively limited to between 90 and 135 minutes of growth, with a mean time of about 110 to 115 minutes. The number of cells began to increase rapidly after 120 minutes, reaching a plateau at 165 minutes. From cell counts it is estimated that 70% to 75% of cells divided during this 45 minute period.

Since the maximum percentage of budded cells counted was only 25%, it must be assumed that not all budded cells were observed. This was possibly due to the depth (0.1 mm) of the counting chamber, which allowed most buds to orient parallel to the optical path of the microscope. However, the counts of budded cells do indicate a phasing of the budding process similar to the more adequately demonstrated phasing of cell division in synchronized cultures. The parameters of the system (duration and timing of lag,

Table 1. Hemocytometer counts of cells, cell groups, and budded cells throughout a synchronous division cycle

	Experiment 1						Experiment 2					
Sample time in minutes	Total cells counted	Cells x 10^7/ml[1]	Cell groups counted[2]	Cell groups x 10^7/ml	Budded cells counted	% budded cells of total cells	Total cells counted	Cells x 10^7/ml[1]	Cell groups counted[2]	Cell groups x 10^7/ml	Budded cells counted	% budded cells of total cells
0	952	1.19	844	1.05	8	0.84	1073	1.35	950	1.19	7	0.65
60	985	1.23	868	1.09	9	1.04	1096	1.37	967	1.21	8	0.73
90	996	1.25	872	1.09	55	5.52	1140	1.43	1001	1.25	66	5.79
105	1020	1.28	904	1.13	228	22.35	1184	1.48	1035	1.29	272	22.97
120	1073	1.34	912	1.14	274	25.54	1208	1.51	1030	1.29	319	26.41
135	1400	1.75	949	1.19	83	5.20	1672	2.09	1115	1.39	87	5.20
150	1503	1.88	1009	1.26	73	4.20	1732	2.17	1145	1.43	71	4.10
165	1549	1.94	1037	1.30	95	9.16	1796	2.25	1176	1.47	161	8.96
180	1564	1.95	1020	1.28	211	13.49	1803	2.25	1165	1.46	230	12.76

[1] Cells x 10^7/ml = cells counted x 1.25 x 10^4

[2] Cell groups is given by the number of single cells counted plus paired cells. This provides a correction necessary to determine plating efficiency (see Appendix 1).

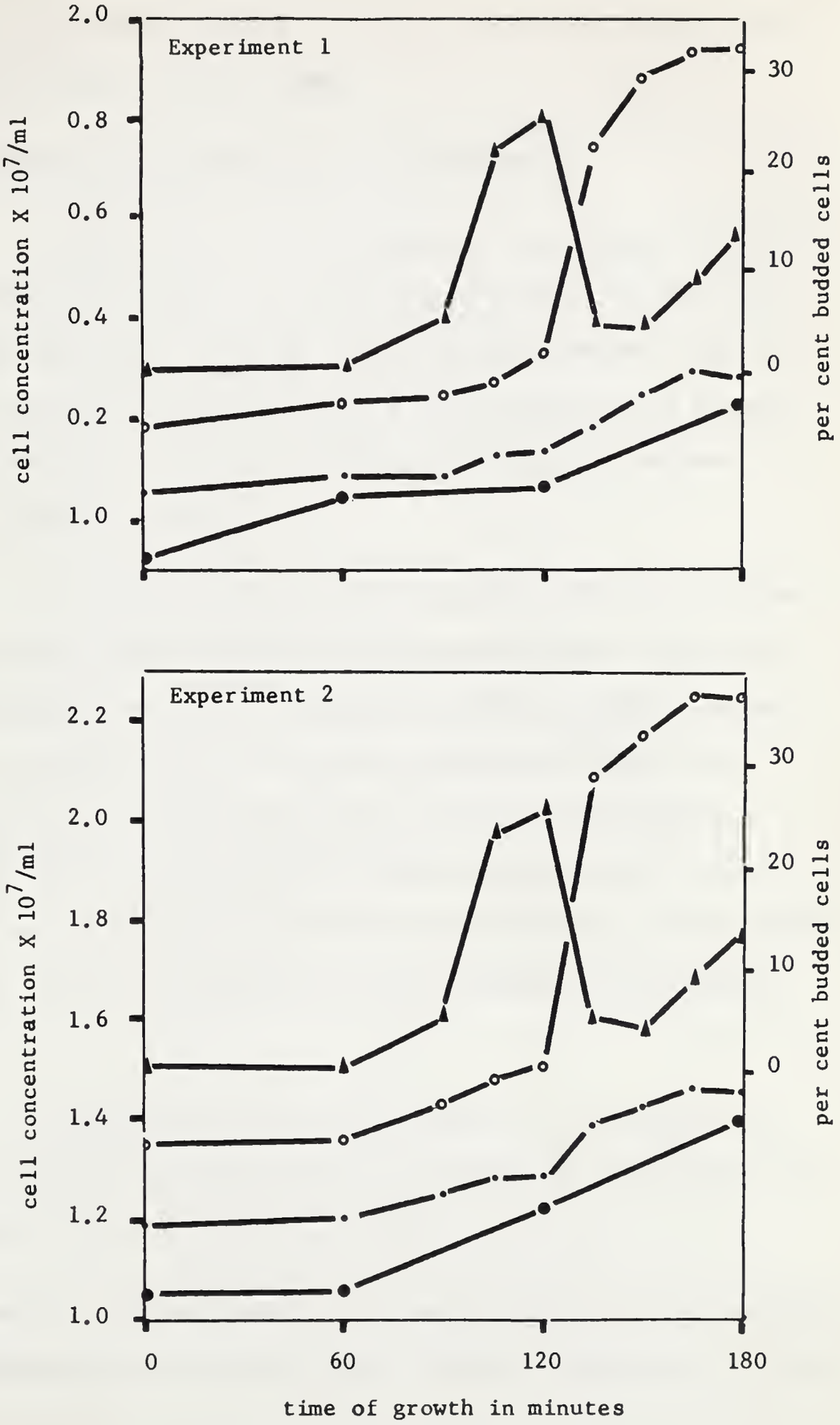
Figure 1. Behavior of a synchronized culture during a single division cycle.

▲—▲ Budded cells, determined by hemocytometer count. Note: Scale is to right.

○—○ Single cells, determined by hemocytometer count.

●—● Cell groups, determined by hemocytometer count.

●—● Cell groups, determined by plate count.


Experiment 1
Experiment 2
cell concentration X 10^7/ml
per cent budded cells
time of growth in minutes
2.0
0.8
0.6
0.4
0.2
1.0
30
20
10
0
2.2
1.8
1.6
1.4
1.2
60
120
180

budding, and division phases; as well as the percentage of dividing cells) are in good agreement with those published by Sylvén et al. (1959) for their prototype system,

II. Survival after Starvation and UV Irradiation

Irradiation of cells brought into synchronous division is expected to demonstrate the relationship between cell stage and irradiation effects. However, the starvation treatment used to induce division synchrony affects cellular metabolism in general, and may itself be expected to affect radiobiological responses of synchronized populations.

Pre-irradiation starvation for both carbon and nitrogen sources was found to markedly decrease survival rate. This is illustrated by Table 2 and by Figure 2, which represent survival after a constant dose of UV given at successive stages in the division cycle which follows removal from starvation medium. Survival at 0 minutes (starved, interdivisional cells) is about 5% of the value at 135 - 165 minutes (non-starved, interdivisional cells). This is in agreement with the findings of Giese et al. (1957).

Superimposed on the greater effect of starvation is a lesser variation in lethal sensitivity dependent on cell stage, as indicated by the following observations:

1. Specific processes marking the onset of active metabolism prior to budding appear to confer slightly increased sensitivity at about

the 45 minute period. The results presented by Williamson and Scopes (1960) and by Williamson (1964) show that during the pre-budding period, oxygen consumption, and total cell nitrogen, protein, and DNA content remain constant; but RNA synthesis begins at a reduced rate.

2. The first budding phase (broadly, 75 - 120 minutes, see Fig. 4), during which DNA synthesis occurs, is associated with a 10 fold increase in survival rate. A large component of this increase is no doubt due to recovery of cells from the starved state. The smaller decrease in survival following passage into the second inter-division stage (120 - 165 minutes) probably more accurately reflects the degree of resistance to lethal effects conferred by the budding state. A similar effect has been well demonstrated in Saccharomyces cerevisiae (Elkind and Sutton, 1959a; Giese et al., 1957) and in Schizosaccharomyces pombe (Swann, 1962).

III. Frequency of UV-induced Mutation

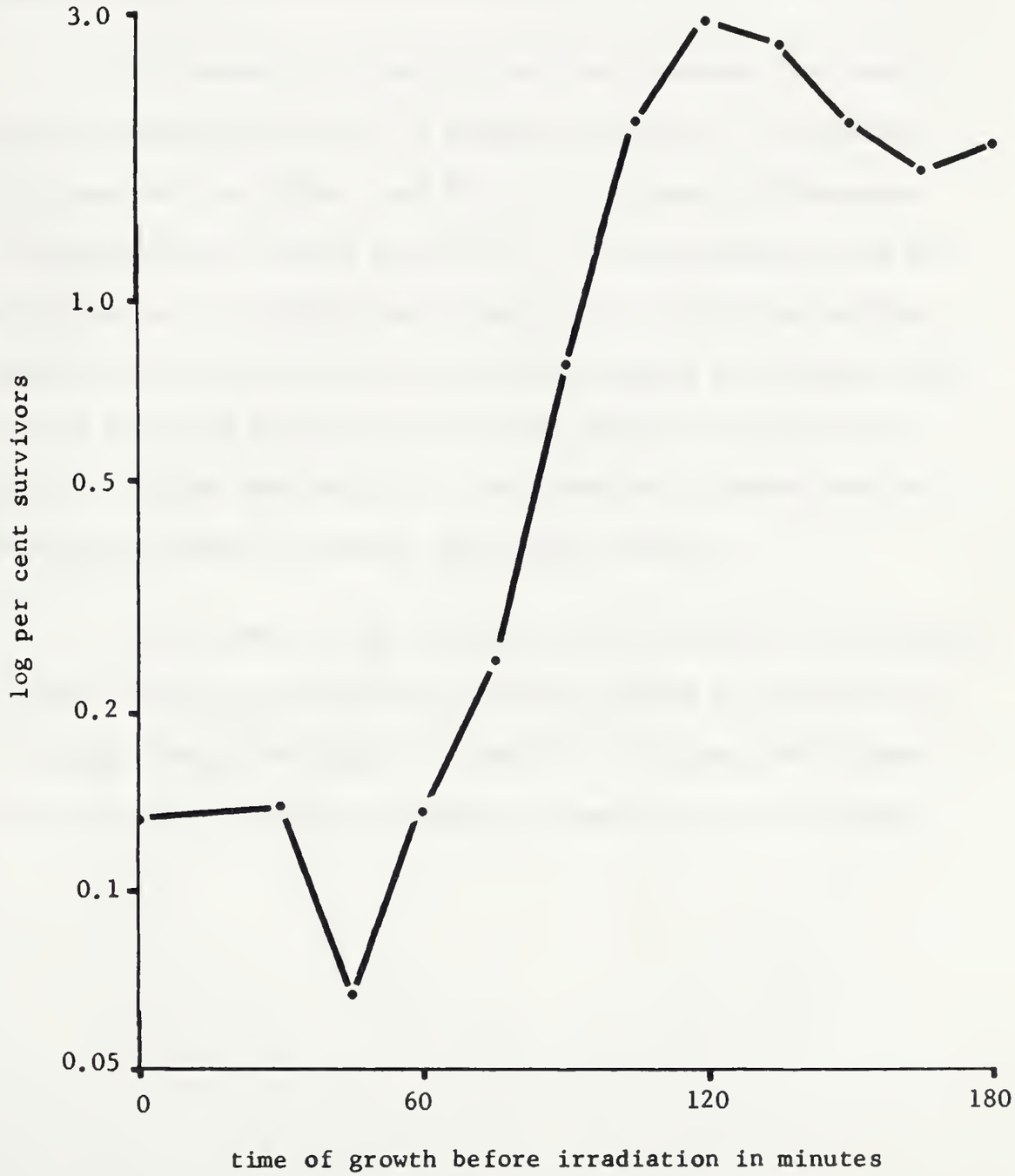
Calculation of mutation frequencies was based on counts of half- and quarter-sectored colonies only. Since mutation from the initial red phenotype resulted in new phenotypes ranging from pink to white, the counting of whole-colony mutants involved classification based on qualitative distinctions which were not considered to be reliable. In contrast, juxtaposition of two color variants in the same colony made identification certain. Detection of sectors smaller than quarter-colony appeared to be dependent on colony size: counts of these classes were disproportionately low in plates with numerous small colonies;

Table 2. Survival after UV irradiation throughout a synchronous division cycle. Calculated from colony counts.

Sample time in minutes	Experiment 1 Av. # colonies/plate[1]		% survival	Experiment 2 Av. # colonies/plate		% survival	Experiment 3 Av. # colonies/plate		% survival	Average % survival
	Control	Irradiated		Control	Irradiated		Control	Irradiated		
	Dil'n:10^{-5}	Dil'n:10^{-2}		Dil'n:10^{-5}	Dil'n:10^{-2}		Dil'n:10^{-5}	Dil'n:10^{-2}		
0	431.0	600.0	0.139	205.3	263.3	0.128	182.2	232.5	0.128	0.132
30	410.3	617.7	0.151	198.0	240.0	0.121	213.0	306.7	0.144	0.139
45	418.5	327.5	0.078	211.0	133.3	0.063	197.7	115.0	0.056	0.066
60	427.3	625.0	0.146	210.3	256.7	0.122	225.3	293.3	0.130	0.137
		Dil'n:10^{-3}								
75	403.0	126.5	0.314	223.3	510.0	0.228	186.7	347.3	0.186	0.243
					Dil'n:10^{-3}			Dil'n:10^{-3}		
90	422.0	303.0	0.718	200.5	166.7	0.831	190.0	140.0	0.737	0.762
		Dil'n:10^{-4}								
105	435.5	94.3	2.165	222.0	400.0	1.802	238.0	470.5	1.977	1.981
120	470.0	145.7	3.100	245.0	613.3	2.503	233.3	753.3	3.229	2.944
135	511.3	126.0	2.464	266.7	723.3	2.712	231.3	640.0	2.767	2.648
150	541.5	83.0	1.533	275.3	660.0	2.397	261.7	533.3	2.038	1.989
165	560.7	75.7	1.350	285.0	503.3	1.766	275.0	475.7	1.730	1.615
180	591.0	87.0	1.472	296.7	566.7	1.910	293.3	586.7	2.000	1.794

[1] The average for each determination is calculated from colony counts of a series of three plates.

Figure 2. Survival after UV irradiation throughout a synchronous division cycle. Average results of three experiments.


3.0
1.0
0.5
0.2
0.1
0.05
log per cent survivors
0
60
120
180
time of growth before irradiation in minutes

therefore they also were excluded from the final data. It should be noted, however, that the curve for total mutants, representing all sector types plus whole-colony variants, is not essentially different in shape from the curve for the selected data (see Appendix 2). Each determination on both curves was based on a minimum of about 1,500 colonies observed.

The frequency of induced mutation increased from about 0.3% with irradiation at 0 - 75 minutes to 3.5% with irradiation at 105 minutes (see Table 3 and Figure 3). Figure 4 illustrates the correspondence between sensitivity to induced mutation and the budding phase. The slight lag in the curve representing budding probably indicates that it is the earliest stages of budding, which are less likely to be observed, that are the most sensitive to mutation. It has been shown that DNA synthesis is restricted to the earliest stages of budding (Williamson, 1965).

The failure of the mutation rate to return to the initial low level during the second inter-division period is possibly due to a slight protective effect of starvation, although this cannot be certain since synchrony has begun to degenerate at this point.

Table 3. UV induced half- and quarter-sectored colonies throughout a synchronous division cycle

Sample time in minutes	Total colonies observed	1/2-sectored colonies	1/4 red-sectored colonies	1/4 white-sectored colonies	Total 1/2- & 1/4-sectored colonies	% 1/2- & 1/4-sectored of total colonies
0	5326	3	3	0	6	0.11
30	2829	4	1	2	7	0.25
45	3314	2	2	6	10	0.30
60	3650	1	1	3	5	0.14
75	3866	10	6	4	20	0.52
90	7555	30	14	7	51	0.68
105	1869	42	16	9	67	3.58
120	1841	24	17	16	57	3.10
135	1883	12	16	11	39	2.07
150	1524	14	6	8	28	1.84
165	1316	15	6	7	28	2.13
180	2119	28	16	15	59	2.78

Figure 3. UV-induced mutation throughout a synchronous division cycle.

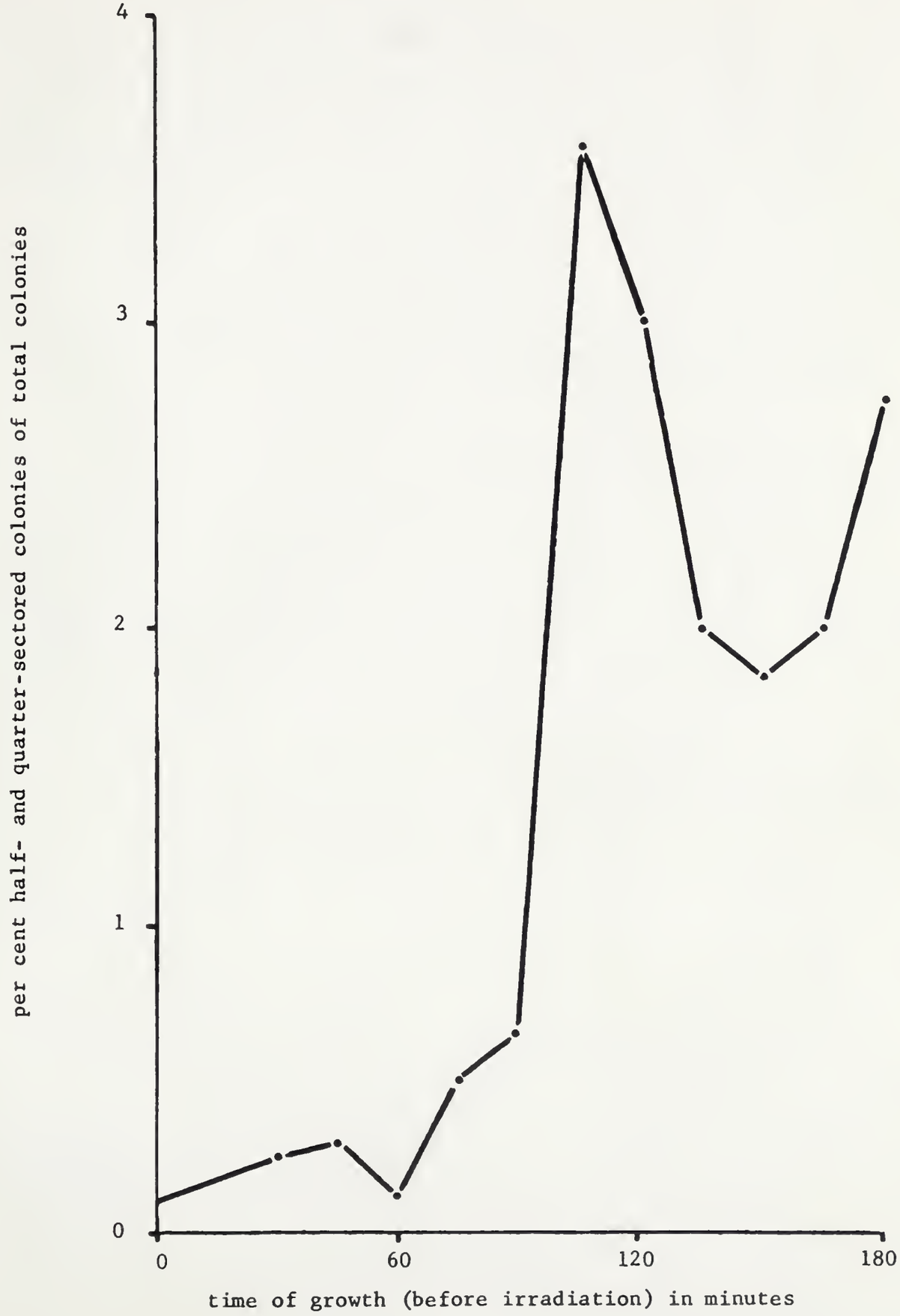
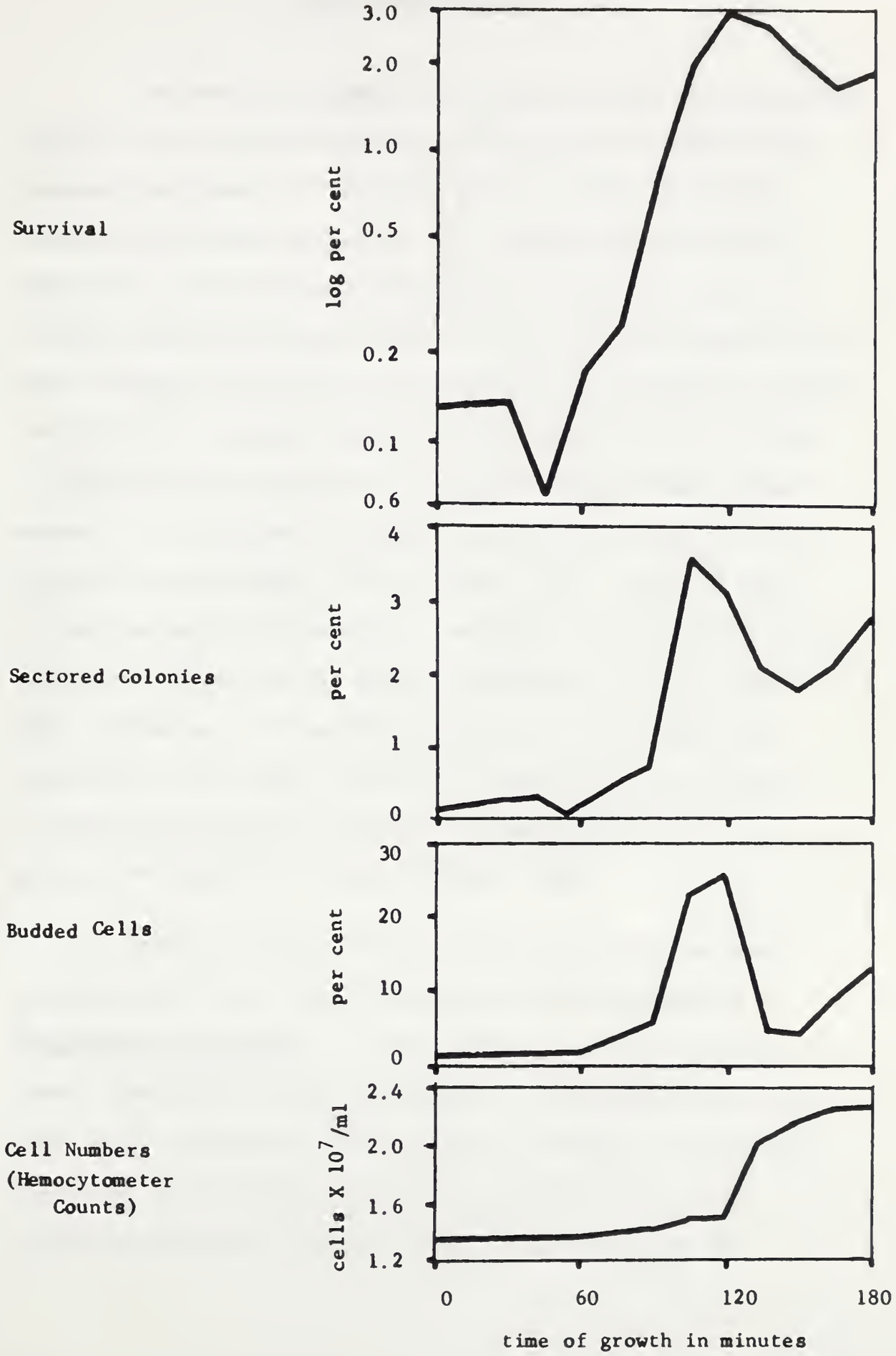

4
3
2
1
0
per cent half- and quarter-sectored colonies of total colonies
0
60
120
180
time of growth (before irradiation) in minutes

Figure 4. Combined results.


Survival
log per cent
3.0
2.0
1.0
0.5
0.2
0.1
0.6
Sectored Colonies
per cent
4
3
2
1
0
Budded Cells
per cent
30
20
10
0
Cell Numbers
(Hemocytometer
Counts)
cells X 10^7/ml
2.4
2.0
1.6
1.2
0
60
120
180
time of growth in minutes

GENERAL DISCUSSION

The results obtained from irradiating cells in synchronized division show that the process of bud formation is associated with increased resistance to UV-induced lethality, and a pronounced increase in UV-induced mutation. It is expected on the basis of target theory that DNA duplication should increase sensitivity to UV damage (James and Werner, 1965). It has also been suggested that cells irradiated during genetic replication will obligatorily express the lethal and mutagenic effects induced, whereas cells irradiated at inter-divisional stages have the opportunity to repair damage before it is stabilized by replication or produces lethality by blocking division (Swann, 1962; Holliday, 1965). Wacker's model of inactivation by intermolecular dimerization during replication similarly predicts that the greatest sensitivity to this lethal effect should occur during DNA synthesis, or at the time of bud formation in this system. These predictions have been confirmed by experiments in several bacterial systems (Wacker, 1963; Ito *et al.*, 1964) and in the fungi (Holliday, 1965).

Since for lethal effects of UV the converse has been demonstrated to be the case by this and other investigations in *Saccharomyces cerevisiae*, it seems probable that some other non-genetic factor(s) is acting to increase UV resistance in dividing yeast cells. Moustacchi (1963) has given evidence to support the hypothesis that budding cells have a specific protein which stabilizes DNA against radiation-induced lesions, either by

reducing energy transfer, or by facilitating repair. It is evident from the results obtained with induced mutation that damage to DNA does occur to a significantly increased extent during budding and DNA synthesis. This result has also been obtained with X-ray mutagenesis (Fritz-Niggli, 1962), and would tend to support the hypothesis of increased efficiency of repair in budding cells. Repair of lethal damage to non-lethal mutations would increase survival and also the apparent rate of induction of mutation.

The seemingly discordant results of Holliday (1965) with synchronized cultures of Ustilago maydis do in fact provide some information which seems to support these findings in yeast. The period of genetic replication in Ustilago is accompanied by an increase in UV-induced lethality and mitotic recombination. However, DNA replication and budding occur during distinctly separate phases in this system, and the budding phase coincides with the period of greatest resistance to UV damage. It seems tenable at this point to conclude that the potentially lethal effects of UV on budding yeast cells are obscured by specific radioresistant properties conferred by the process of cell-division. Mutation frequency, which directly reflects damage to DNA, remains positively correlated with the period of genetic replication as predicted.

Reduced survival produced by pre-irradiation starvation may also be related to the ability of cells to repair UV damage, since it is selectively effective on budding cells, and specifically associated with an organic nitrogen deficiency (Moustacchi, 1963; Giese et al., 1957).

Some information about the mechanism of induction of sectored colonies may be inferred from this study. If, as suggested by Yamasaki et al. (1964) and Ito et al. (1964), the production of fractional- and whole-colony variants is a function of the number of strands of DNA receiving a mutational hit, the relative frequencies of these events should reflect the strandedness of the DNA at the time of irradiation. The data presented give no evidence of a consistent relationship between cell stage and type of mutation induced. The distribution is apparently independent of division stage.

This negative finding thus tends to support alternate hypotheses such as: 1. delayed action of UV through RNA precursors (Doudney, 1961); 2. delay or failure in recovery of one or more cells of a microcolony in early divisions following recovery (James, 1954); 3. the induction of heterozygosity in DNA (Whitehouse, 1965) which is repaired in the first few divisions after irradiation; 4. multistrandedness of chromosomes (Swann, 1962).

The possibility that sectoring is the result of segregating cytoplasmic factors is eliminated by the observation that all sectors originate at the centre of a colony, and the finding that similar induced mutations in the same genetic strain are stable on transfer (Roman, 1956; Ito et al., 1964).

SUMMARY AND CONCLUSIONS

1. Synchronized cultures of a haploid, adenine-red strain of Saccharomyces cerevisiae were used to study the effect of starvation and cell stage on UV-induced lethality and mutation affecting pigment production.

2. Pre-irradiation starvation markedly reduced survival, but produced little or no effect on mutation rate. The mechanism implicated is a reduction in the effectiveness of repair processes.

3. Cell stage at irradiation was associated with a lesser variation in survival rate. Budding cells were most resistant; interdivisional cells least resistant. It is suggested that physiological conditions characteristic of budding cells confer resistance to, or facilitate repair of anticipated lethal effects of UV on replicating DNA in budding cells.

4. Cell stage at irradiation greatly influenced mutability. Mutation was induced in budding cells at about 10 times the rate induced in interdivisional cells. This result is correlated with the inferred time of genetic replication and with predictions based on known mechanisms of action of UV radiation on DNA. The possibility is suggested that the same mechanism operating to increase survival in budding cells may enhance the apparent mutation rate by repairing lethal mutations to non-lethals.

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APPENDICES

APPENDIX 1

TYPICAL RESULTS OF A PLATING EXPERIMENT: DETERMINATION OF PLATING EFFICIENCY THROUGHOUT A SYNCHRONIZED DIVISION CYCLE

Comparison of cell groups per ml determined by colony counts and by hemocytometer counts to obtain plating efficiency

Sample time in minutes	Plate counts (dil'n.x10^{-5}) a	b	c	Total colonies counted	Average number colonies	Cell gps x10^7/ml (from colony count)	Cell gps x10^7/ml (from hemocytometer count)[1]	Plating efficiency as %[2]
Experiment 1								
0	81	97	95	273	91.00	0.91	1.05	86.67
60	105	112	98	315	105.00	1.05	1.09	96.33
120	101	102	118	321	107.00	1.07	1.14	93.86
180	128	118	124	370	123.33	1.23	1.28	96.09
Experiment 2								
0	100	104	112	316	105.33	1.05	1.19	88.24
60	106	110	122	338	112.67	1.13	1.21	93.39
120	131	123	112	366	122.00	1.22	1.29	94.57
180	142	129	148	419	139.67	1.40	1.46	95.89

[1] See Results and Discussion, Table 1.

[2] Plating efficiency = $\frac{\text{Hemocytometer count of cell groups/ml}}{\text{Colony count of cell groups/ml}}$

APPENDIX 2

COMPLETE DATA FOR EXPERIMENTS ON UV-INDUCED MUTATION

UV-induced whole- and fractional-colony mutations

Sample time in minutes	Total colonies observed	Wholly white colonies		Sectored colonies						Total mutants	
				Total sectored colonies		1/2-plus 1/4-sectors		Less than 1/4-colony sectors			
		No.	% of total colonies	No.	% of total colonies	No.	% of total colonies	No.	% of total colonies	No.	% of total colonies
0	5326	2	0.04	9	0.17	6	0.11	3	0.06	11	0.21
30	2829	12	0.42	9	0.32	7	0.25	2	0.07	21	0.74
45	3314	11	0.33	17	0.51	10	0.30	7	0.21	28	0.84
60	3650	12	0.33	6	0.16	5	0.14	1	0.03	18	0.49
75	2866	15	0.39	29	0.75	20	0.52	9	0.31	44	1.54
90	7555	13	0.17	62	0.82	51	0.68	11	0.15	75	0.99
105	1869	33	1.77	75	4.01	67	3.58	8	0.43	108	5.78
120	1841	46	2.50	70	3.80	57	3.10	13	0.71	116	6.30
135	1883	33	1.75	49	2.60	39	2.07	10	0.53	82	4.35
150	1524	33	2.17	39	2.56	28	1.84	11	0.72	72	4.72
165	1316	23	1.75	31	2.36	28	2.13	3	0.23	54	4.10
180	2119	32	1.51	63	2.97	59	2.78	4	0.19	95	4.48

APPENDIX 2. Distribution of whole-colony and fractional-colony mutants.

▼—▼ total mutants

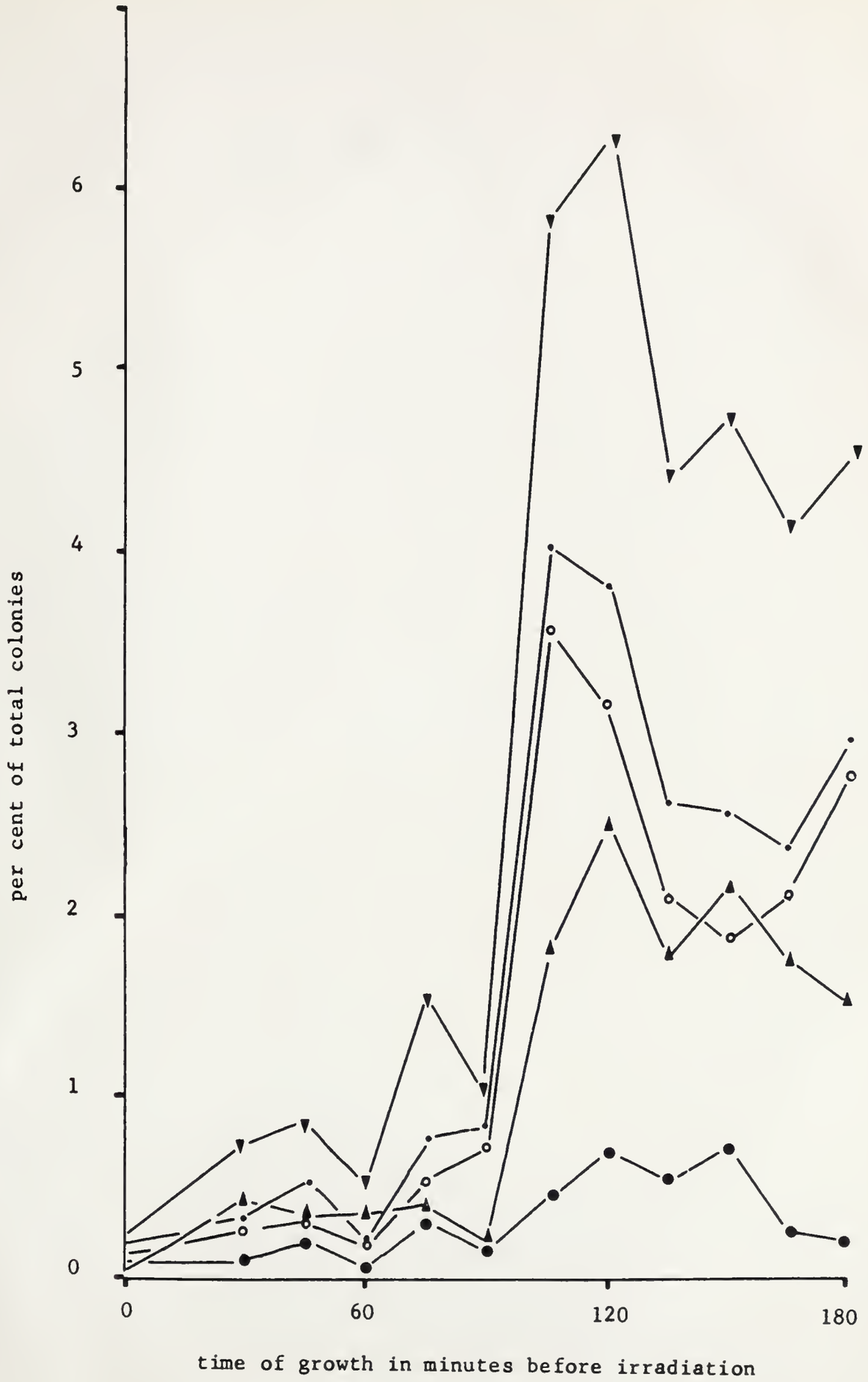
∘—• all fractional-colony mutants

o—o 1/2-colony plus 1/4-colony mutants

▲—▲ whole-colony mutants

●—● less than quarter-colony mutants

Note: The curves for whole-colony and less than quarter-colony mutants show a large component of erratic variability. This data has been considered unreliable because of limitations inherent in the experimental technique by which it was obtained (see Results and Discussion, III). The data judged to be reliable are represented in the curve for 1/2-colony and 1/4-colony mutants. This curve shows consistent trends which are essentially the same as those present in the curve for total mutants, but indicates a reduction in the variability due to improved technical procedures.


per cent of total colonies
0
1
2
3
4
5
6
0
60
120
180
time of growth in minutes before irradiation

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